Ruxolitinib Attenuates Cutaneous Lupus Development in a Mouse Lupus Model

Journal of Investigative Dermatology (2015) **135,** 1912–1915; doi:10.1038/jid.2015.107; published online 9 April 2015

TO THE EDITOR

Systemic lupus erythematosus (SLE) is an autoimmune disease that is classically associated with fatigue, fever, joint pains, and skin involvement, although any organ system can be affected. Almost all patients with SLE experience cutaneous manifestations at some point in their disease course, and an additional population of patients exists who experience cutaneous lupus but do not meet the criteria for SLE. Discoid lupus erythematosus (DLE), a common lupus-specific skin manifestation, is often a source of disfigurement and scarring alopecia. Currently, there are no Food and Drug Administration (FDA)–approved therapies for DLE. Treatment for DLE is empiric; DLE is treated with broad-spectrum immunosuppressants that have the potential for deleterious side effects and often require frequent monitoring. There is therefore an unmet need for effective treatments for DLE with favorable side-effect profiles (Jessop *et al.,* 2000). The IFN signature has been described in both SLE and in cutaneous lupus, inviting studies targeting this pathway for therapeutic purposes.

Given the significantly increased IFN signature in human DLE lesions (Jabbari *et al.,* 2014), we hypothesized that blocking proximal signaling molecules downstream of the type I and II IFN receptors may attenuate the disease. Ligation of these receptors results in the activation of JAK1 and TYK2 or the activation of both JAK1 and JAK2 (for the type I IFN receptor or the type II IFN receptor, respectively) (Platanias, 2005). Ruxolitinib is a small-molecule tyrosine kinase inhibitor with relative specificity for JAK1 and JAK2, approved in 2012 for the treatment of myelofibrosis (Harrison *et al.,* 2012; Verstovsek *et al.,* 2012). Ruxolitinib was administered by oral gavage to female MRL/lpr mice, prone to spontaneously develop cutaneous and systemic lupus, before development of severe skin involvement. This treatment indeed attenuated the development of severe skin lesions by the MRL/lpr mice (Figure 1a). Ruxolitinib-treated mice exhibited significantly reduced lesion severity scores by week 4 of treatment compared with control treatment (Figure 1b). Histopathologic analysis showed a significant reduction in epidermal hyperplasia and inflammatory infiltrate with ruxolitinib treatment (Figure 1c and d).

The MRL/lpr mouse model system also exhibits other manifestations of SLE including autoantibody production, renal disease, and immune complex deposition. Assessments of renal endocapillary proliferation, crescent formation, and interstitial inflammation did not identify significant differences between ruxolitinib and vehicle control–treated mice (Figure 1e). Immune complex deposition in kidneys was also unchanged with ruxolitinib treatment as assessed by staining for IgG and C3 (Figure 1e). Examinations of other lupus manifestations did not detect any change with ruxolitinib treatment. Autoantibody levels (Supplementary Figure S1 online), lymphadenopathy, and splenomegaly (Supplementary Table S1 online) showed no consistent differences between groups. In sum, only the skin involvement was significantly alleviated in mice that received ruxolitinib compared with those that received vehicle control.

Prevention of the development of cutaneous manifestations of lupus with continued progression of other disease manifestations was surprising. One hypothesis that may account for this difference pertains to the timing of drug administration. Ruxolitinib treatment was started when the animals started to develop the first signs of skin disease and, at this point, had already exhibited appreciable gross lymphadenopathy, renal disease, and autoantibody formation. Ruxolitinib administration may have been able to curtail these extracutaneous markers of end-organ damage if it had been initiated at an earlier age. Alternatively, a distinct pathogenic mechanism may be responsible for the cutaneous lupus as opposed to lupus nephritis. Indeed, it seems likely that IL-17 is a contributor to the pathogenesis of lupus nephritis (Crispín *et al.,* 2008; Zhang *et al.,* 2009), whereas it is likely playing a very limited role in discoid lupus (Jabbari *et al.,* 2014). Further studies will therefore be required to distinguish these possibilities.

In order to examine the effects of ruxolitinib on the IFN response, MRL/lpr splenocytes were cultured with IFN-α and -γ along with graded concentrations of ruxolitinib. After 6 hours of coculture, expression of IFN signature genes was assessed (Figure 2 and Supplementary Figure S2 online). Ruxolitinib diminished the expression of IFN response genes (Figure 2a). In particular, the T-cell chemokines Cxcl9 and Cxcl10, both of which exhibited relatively high upregulation in response to IFNs, were abrogated in expression in the presence of ruxolitinib. Ruxolitinib therefore decreases the production of chemotactic signals for T cells.

An assessment of the T-cell infiltrate was performed by counting immunofluorescently stained $CD3^+$, $CD4^+$, and $CD8^+$ cells (Figure 2b and c). The skin from ruxolitinib- versus vehicle-treated mice had significantly fewer $CD3^+$ cells and $CD4^+$ cells. CD8 T cells were a minor part of the immune infiltrate, but the cumulative data did show

Abbreviations: DLE, discoid lupus erythematosus; FDA, Food and Drug Administration; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription

Accepted article preview online 19 March 2015; published online 9 April 2015

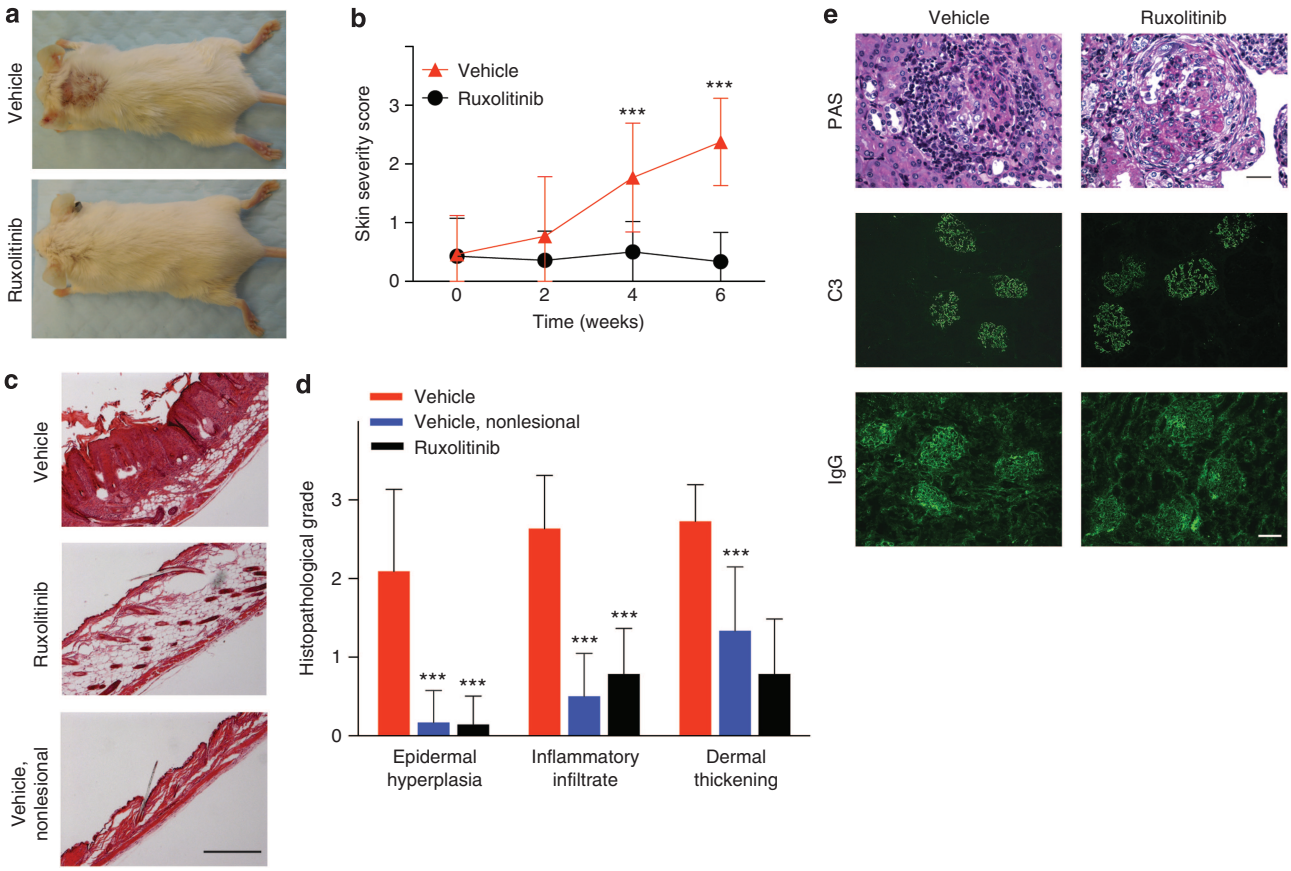

Figure 1. JAK inhibition prevents the development of cutaneous lupus lesions. (**a**) Gross skin lesions after control vehicle or ruxolitinib treatment. (**b**) Gross skin lesions were graded as described in the Supplementary Materials and Methods online biweekly starting at initiation of treatment (week 0), and trend was analyzed by mixed modeling. ***$P<0.001$. (**c**) Periodic acid Schiff (PAS)–stained skin sections are shown for lesional and nonlesional skin in vehicle-treated mice and representative skin from ruxolitinib-treated mice. Black bar = 100 μm. (**d**) Grading was performed as indicated in the Supplementary Materials and Methods online for epidermal hyperplasia, inflammatory infiltrate, and dermal thickening, and is shown by the group and analyzed by ordinal regression versus control lesional skin. ***$P<0.001$. (**e**) Kidneys were harvested at the end of treatment and stained as indicated and described in the Supplementary Materials and Methods online. Top panels, black bar = 20 μm. Bottom panels, white bar = 50 μm.

differences between these groups. These results indicate that ruxolitinib was diminishing the immune infiltrate in cutaneous lupus lesions, likely in part by diminishing the expression of T-cell chemotactic genes.

In sum, the use of a small-molecule inhibitor of JAK1 and JAK2, the proximal signaling molecules that transduce the type I and II IFN signal, attenuated the development of cutaneous manifestations in a mouse model of lupus. Interestingly, other manifestations of SLE were not significantly affected, and a striking specificity for alleviating the development of skin lesions was observed. Previously published data examining the effects of ablating type I and II IFN signaling are mixed in their effects on SLE manifestations (Crow, 2014), although skin manifestations have not always been well described in prior studies. Depending on the mouse model used, genetic deficiency of the type I IFN receptor either alleviated (Braun and Demengeot, 2003; Santiago-Raber *et al.,* 2003) or exacerbated (Hron and Peng, 2004) SLE manifestations. Type II IFN signaling attenuation, either by genetic ablation (Peng *et al.,* 1997; Hron and Peng, 2004) or by blocking antibodies (Jacob *et al.,* 1987), delayed or prevented SLE manifestations and, in one study, seemed to mitigate the exacerbated phenotype seen due to type I IFN receptor ablation (Hron and Peng, 2004). Much like this last study, we show that the net effect of inhibiting both pathways with ruxolitinib resulted in a favorable response in a lupus end organ, in this case the skin. Furthermore, the use of a small-molecule inhibitor offers advantages not currently possible for antibody treatments including adaptation into a topical form (Fridman *et al.,* 2011). A double-blind clinical trial testing the efficacy of a topical form of ruxolitinib has been shown to have some efficacy in treating lesions of psoriasis (Punwani *et al.,* 2012), an autoimmune skin disease in which IFNs contribute to its pathogenesis (Lowes *et al.,* 2014).

The MRL/lpr mouse model is notable for its spontaneous development of cutaneous manifestations of disease (Ghoreishi and Dutz, 2010). Although the MRL/lpr model exhibits many of the features of human cutaneous lupus (Furukawa *et al.,* 1984; Kanauchi *et al.,* 1991; Furukawa, 1997), not all aspects of the human condition are replicated, most notable of which may be the lack of an interface dermatitis as a predominant histological feature in

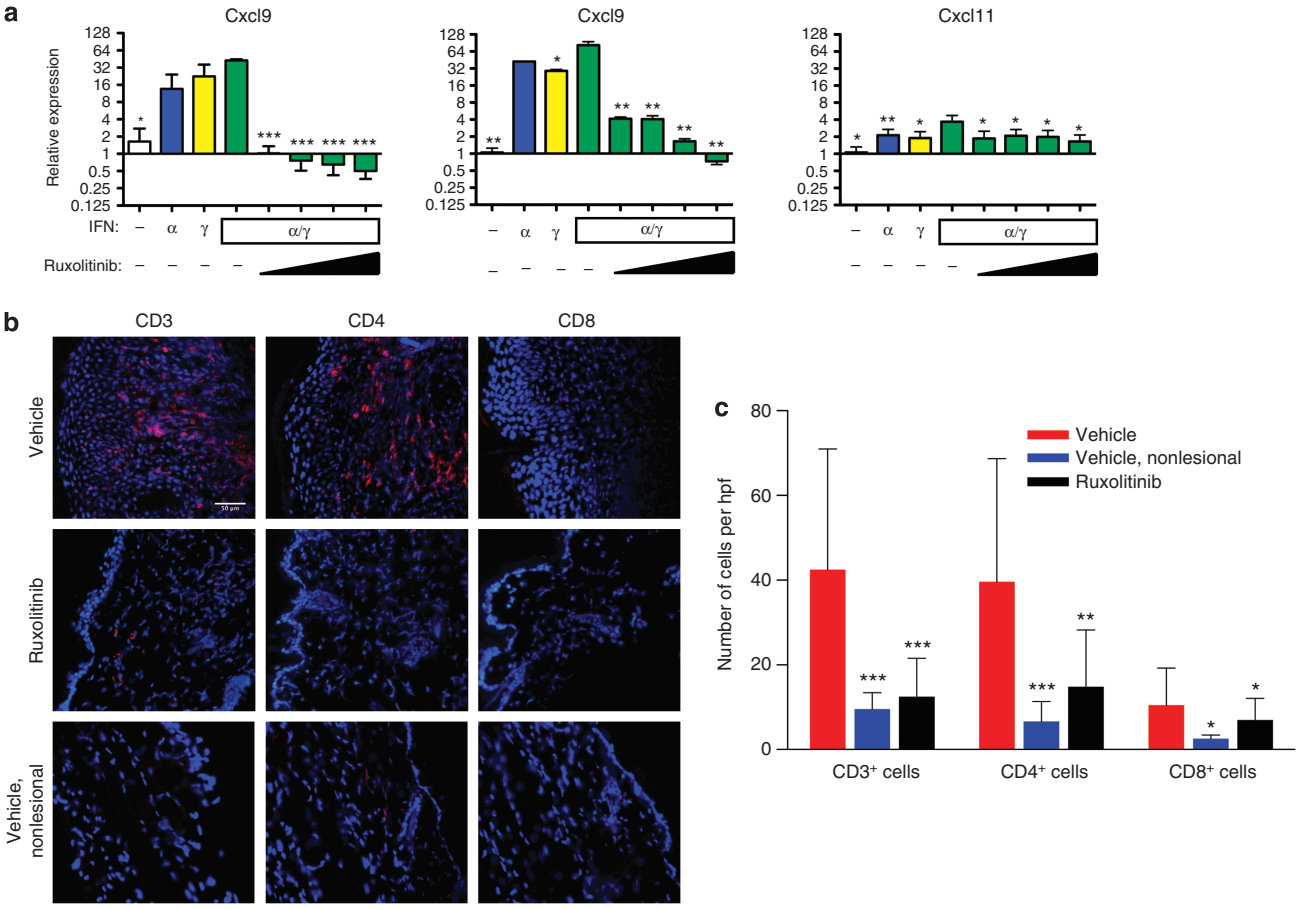

Figure 2. Ruxolitinib treatment decreases T-cell chemokine production *in vitro* and decreases T-cell infiltration *in vivo*. (**a**) Reverse transcriptase–PCR (RT-PCR) was performed for the indicated chemokines on MRL-lpr splenocytes cocultured with the indicated IFNs and graded concentrations of ruxolitinib (10, 30, 100, or 300 nM) for 6 hours. Relative fold changes are shown on the y axis. $*P<0.05$, $**P<0.005$, and $***P<0.001$ compared with coculture with IFN-α and IFN-γ together. (**b**) Skin sections from mice treated with ruxolitinib or vehicle control (lesional and nonlesional) were stained with α-CD3, α-CD4, and α-CD8 as indicated and described in the Supplementary Materials and Methods online. DAPI (4',6-diamidino-2-phenylindole) was used for counterstaining. (**c**) Positive staining cells per high-power field (hpf) were counted from skin sections stained with α-CD3, α-CD4, or α-CD8. $*P<0.05$, $**P<0.005$, and $***P<0.001$.

the MRL/lpr model. Further investigations will therefore be required before large clinical trials addressing the efficacy of ruxolitinib in human cutaneous lupus.

The findings here show that JAK inhibition prevents the development of cutaneous lupus, supporting an important role of JAK signaling in cutaneous lupus pathogenesis with a seemingly diminished role in the pathogenesis of dysfunction in other lupus end organs. Greater elucidation of the role of IFN in cutaneous lupus and DLE development, as well as the role of JAK/signal transducer and activator of transcription (STAT) inhibition, has the potential to identify new treatments for human DLE, and our findings identify JAK1/2 inhibition as a therapeutic strategy worthy of further studies.

The Materials and Methods are documented in the Supplementary Data online. The institutional animal care and use committee at the Columbia University Medical Center approved all described studies.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Emily Chang and Ming Zhang for excellent technical support and Ryan Langlois for helpful discussions. AJ was supported by a Physician-Scientist Career Development Award from the Dermatology Foundation and also by the Louis V. Gerstner, Jr Scholars Program.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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SIRT1 Activation Ameliorates Aldara-Induced Psoriasiform Phenotype and Histology in Mice

Journal of Investigative Dermatology (2015) **135,** 1915–1918; doi:10.1038/jid.2015.82; published online 2 April 2015

TO THE EDITOR

Sirtuin 1 (SIRT1), an NAD^+-dependent deacetylase (Imai *et al.*, 2000), acts as a metabolic sensor that functions on both histone and non-histone proteins (Leibiger and Berggren, 2006; Li, 2013). In this study, we find that SIRT1 activation in animals could inhibit Aldara-induced psoriasiform lesions. Moreover, SIRT1–STAT3 interaction may serve as an important mechanism that underlies this anti-psoriasis process in keratinocytes.

A psoriatic mouse model (The protocols were approved by the Animal Care and Use Committee at Nanjing University) using Aldara cream (5% imiquimod, 3 M Pharmaceuticals) on shaved back skin exhibited signs of erythema, scaling, and thickening, a psoriasiform phenotype (van der Fits *et al.*, 2009; Walter *et al.*, 2013). Histologic examination showed epidermal hyperplasia and parakeratosis (Figure 1a and b). In addition, the marker for cellular proliferation Ki-67 and the marker for abnormal differentiation of keratinocytes Keratin 17 (Fu and Wang, 2012) were significantly increased in the lesion epithelia (Figure 1d and e). The manifestations closely resembled the characteristics of psoriatic pathology (Supplementary Figure S1 online).

Interestingly, the severity of the skin lesion was significantly reduced, when the mice were treated with an SIRT1 activator resveratrol, before and during Aldara administration. This resulted in smoother and thinner skins with decreased scales and erythemas, compared with the mice treated with Aldara only. To determine whether SIRT1 functioned in this process, a SIRT1 inhibitor EX527 was applied to the mice in the same manner. EX527 treatment exacerbated the psoriasiform symptoms (Figure 1a). The score of psoriasis area and the severity index showed a consistent change (Figure 1c). Histologically, skin lesions of resveratrol-treated mice showed reduced epidermal hyperplasia. In comparison, increased epidermal hyperplasia and acanthosis were observed in EX527-treated mice (Figure 1b, Supplementary Figure S2 online). The changes in Ki-67 and Keratin 17 levels in the epithelia were consistent with the histological alterations (Figure 1d and e, Supplementary Figure S3A–S3C online). Furthermore, increased $CD4^+$ immunocyte infiltration was observed in the Aldara-induced lesional skins, which resembled one of the characteristics of human psoriatic skin tissues. The infiltration of $CD4^+$ immunocytes was reduced in the resveratrol group and increased in the EX527 group (Figure 1f).

Signal transducer and activator of transcription 3 (STAT3) is a latent cytoplasmic transcription factor that regulates cell growth and differentiation in response to cytokines (Sano *et al.*, 2008). Excessive activation of STAT3

Abbreviations: Ac-STAT3, acetylated STAT3; PY-STAT3, STAT3 phosphorylated in Tyr705; SIRT1, sirtuin 1; STAT3, signal transducer and activator of transcription 3

Accepted article preview online 3 March 2015; published online 2 April 2015